


A randomized controlled trial: branched-chain amino acid levels and glucose metabolism in patients with obesity and sleep apnea

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SUMMARY

There is evidence that changes in branched-chain amino acid (BCAA) levels may correlate with the efficacy of therapeutic interventions for affecting improvement in metabolic control. The objective of this study was to evaluate whether serum concentrations of BCAAs (leucine, isoleucine, valine) could mediate in insulin sensitivity and glucose tolerance after continuous positive airway pressure (CPAP) treatment in patients with obstructive sleep apnea (OSA). A prospective randomized controlled trial of OSA patients with morbid obesity was conducted. Eighty patients were randomized into two groups: 38 received conservative treatment and 42 received CPAP treatment for 12 weeks. Plasma levels of BCAA, glucose tolerance and insulin resistance were evaluated at baseline and after treatment. After treatment, significant decreases of leucine levels were observed in both groups when compared with baseline levels ($P < 0.005$). With respect to patients with normal glucose tolerance (NGT), patients with impaired glucose tolerance (IGT) had higher baseline levels of isoleucine (78 ± 16 versus $70 \pm 13 \mu\text{mol L}^{-1}$, $P = 0.014$) and valine (286 ± 36 versus $268 \pm 41 \mu\text{mol L}^{-1}$, $P = 0.049$), respectively. Changes in levels of leucine and isoleucine after treatment were related negatively to changes in fasting plasma glucose and glycosylated haemoglobin values only in the conservative group ($P < 0.05$). In summary, we found that the treatment with CPAP for 12 weeks caused similar changes in circulating BCAAs concentrations to conservative treatment and a differential metabolic response of CPAP and conservative treatment was observed between the relationship of BCAAs and glucose homeostasis. Additional studies are needed to determine the interplay between branched-chain amino acids and glucose metabolism in patients with sleep apnea.

INTRODUCTION

Despite increasing evidence that obstructive sleep apnea (OSA) may impact upon metabolic syndrome or some of its components, independently of obesity, the underlying mechanistic links between OSA and metabolic disturbances have not been well delineated (Bonsignore and Eckel, 2009; Kent *et al.*, 2015).

Recent metabolic profiling of plasma amino acids has revealed a consistent pattern, especially of branched-chain amino acid (BCAA) profiles associated with multiple presentations of metabolic diseases, including obesity and insulin resistance (Cheng *et al.*, 2012; Giesbertz and Daniel, 2016). Dysregulation in BCAA metabolism has been detected in obesity (Newgard, 2012; Shah *et al.*, 2012), and recent studies revealed significant positive associations between circulating BCAAs (leucine, isoleucine, valine) and insulin resistance. Moreover, the plasma concentrations of BCAAs have been involved in the risk of developing type 2 diabetes mellitus and are a prognostic factor of improvement in insulin sensitivity after therapeutic interventions (Laferrere *et al.*, 2011; Magkos *et al.*, 2016; Wang *et al.*, 2011).

Studies addressing the effect of continuous positive airway pressure treatment (CPAP) on glucose metabolism have reported conflicting results (Coughlin *et al.*, 2007; Harsch *et al.*, 2004; Pepin *et al.*, 2012; Weinstock *et al.*, 2012; West *et al.*, 2007). In a recent randomized controlled trial we demonstrated that CPAP treatment in subjects with both morbid obesity and severe sleep apnea improve glucose tolerance without concomitant changes in the homeostasis model assessment–insulin resistance (HOMA-IR) index (Salord *et al.*, 2016). As the HOMA-IR index largely reflects hepatic insulin resistance, these results suggest a potential role of CPAP treatment in peripheral insulin resistance.

In this context, we hypothesized that changes in BCAA metabolism could mediate in the improvement in insulin sensitivity and glucose tolerance after CPAP treatment. To test this hypothesis, we have measured a profile of amino acids to gain an understanding of the differential metabolic response to 12 weeks of CPAP treatment compared to conservative (CT) in a population with morbid obesity and severe OSA without clinically overt diabetes.

METHODS

This is an ancillary study of a prospective, randomized controlled trial (RCT) designed to address whether continuous positive airway pressure treatment improved glucose metabolism in severe OSA patients with morbid obesity without diabetes. The study protocol and study flowchart has been reported previously (Salord *et al.*, 2016).

Trial design

A parallel RCT comparing 12 weeks of CPAP treatment to CT was designed.

Participants and study settings

Patients included in the obesity surgery programme were studied prospectively in two referral sleep clinics in Barcelona from January 2009 to July 2011. Inclusion criteria for the obesity surgery programme were: age between 18 and 65 years; a body mass index (BMI) $\geq 40 \text{ kg m}^{-2}$ or BMI $\geq 35 \text{ kg m}^{-2}$ with comorbidity related to obesity (hypertension, heart disease, degenerative osteoarthritis and respiratory complications). Eligible patients with apnea hypopnea index (AHI) > 30 episodes h^{-1} after a full overnight polysomnography were randomized to receive individualized lifestyle counselling therapy plus CPAP (CPAP group) or conservative treatment (CT group). The exclusion criteria were: current or previous CPAP treatment, previously known diabetes mellitus or diabetic treatment, unstable cardiovascular conditions, severe cognitive or psychiatric disorders, chronic obstructive pulmonary disease, pregnancy, past or current history of alcohol abuse, refusal to participate, disabling daytime sleepiness, professional drivers or professionals performing potentially dangerous activities.

Interventions and protocol

Included patients were randomized to receive individualized lifestyle counselling therapy plus CPAP (CPAP group) or conservative treatment (CT group) consisting only of individualized lifestyle counselling therapy. At baseline and after 12 weeks, each participant completed a detailed questionnaire on medical history, cardiovascular risk factors and current medication. Exercise level and sleep duration were recorded in a self-administered International Physical Activity Questionnaire (IPAQ) and a sleep diary for 15 consecutive days.

In order to aim for a weight loss of 10% of initial weight, the calorie goals during the preparation for bariatric surgery were $1200 \text{ kcal day}^{-1}$ for women and $1500 \text{ kcal day}^{-1}$ for men. These goals were reduced to $1000\text{--}1200 \text{ kcal day}^{-1}$, respectively, if participants did not lose weight satisfactorily. The composition of the diet was structured to enhance glycaemic control and to minimize cardiovascular risk factors. The recommended diet was based on the guidelines of the American Dietitians Association (Raynor and Champagne, 2016) and included a maximum of 30% of total calories from total fat, a maximum of 10% of total calories from saturated fat and a minimum of 15% of total calories from protein. Patients were recommended to increase their intake of fruits, vegetables, poultry, fish and lean meat and limiting dairy fats, fatty meat, sweets, pastries and desserts. The physical activity prescription was 175 min per week of moderate or intensity activity, such as mild or brisk walking, depending on the patient's condition.

The nutritionist provided face-to-face counselling individually four times during the waiting-list period, and at each individual visit patients were asked to bring a 3-day food diary and were asked about the lifestyle changes he or she had

made. Compliance with the diet and exercise was monitored by these individual interviews by the nutritionist.

Excessive daytime sleepiness was quantified by the Epworth Sleepiness Scale. Anthropometric characteristics included BMI, neck circumference, waist circumference, waist/hip ratio and percentage of body fat mass measured by electrical bioimpedance (BIA 101; Akern Bioresearch, Florence, Italy). Clinical blood pressure (BP) was measured according to Spanish guidelines.

Ethical issues

After the indication for bariatric surgery, patients generally spend more than 1 year on the waiting-list for surgery. During this time, patients receive medical care from an endocrinologist and respiratory and sleep studies are performed in order to reduce the peri-operative complications associated with untreated sleep apnea. For ethical reasons, patients were included into our study after the first evaluation by the endocrinologist, and were prioritized for overnight polysomnography (PSG) in order to avoid a delay at the beginning of treatment. At the end of the 12-week study period, CPAP treatment was initiated in all CT patients.

The study protocol was approved by both Ethical Committees: Comité Ético de Investigación Clínica de La Fundació de Gestió Sanitària del Hospital de la Santa Creu i Sant Pau de Barcelona and Comité Ético de Investigación Clínica del Hospital Universitario de Bellvitge (PR052/08, 07/064/797). All participants gave their informed written consent. The trial registration number in the ClinicalTrials.gov was NCT 01029561.

Definitions

At baseline and after 12 weeks of CPAP or CT, a venous blood sample was obtained from all patients in fasting conditions.

Glycosylated haemoglobin (HbA1c), insulin and fasting plasma glucose (FPG) were determined using standard laboratory methods. In patients with FPG <6.7 mmol L⁻¹, plasma glucose measurements were obtained 5 min before and 2 h after administration of 75 g oral glucose (2-h PG). Based on the results of the oral glucose tolerance test (OGTT), normal glucose tolerance (NGT) was defined as 2-h PG <7.8 mmol L⁻¹, impaired glucose tolerance (IGT) as 2-h PG from 7.8 to 11.0 mmol L⁻¹ and diabetes as 2-h PG ≥ 11.1 mmol L⁻¹. Insulin resistance was estimated using the HOMA-IR (Matthews *et al.*, 1985).

Metabolic syndrome (MetS) was defined in accordance with the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) modified criteria (Alberti *et al.*, 2009).

OSA was determined by a full overnight PSG. CPAP titration was performed by an overnight PSG with manual CPAP titration or by autotitration devices. Objective treatment compliance was determined by dividing the number of hours recorded by the CPAP device's built-in hour meter by all

nights during the study period. Patients with an average use time <4 h per night were considered non-compliant.

Amino acid measurements

Analysis of serum samples was conducted in the Biochrom 30+ chromatographic analyser (Biochrom, Cambridge, UK). In brief, samples were deproteinized and amino acids were analysed by high-performance liquid chromatography and spectrometric detection. The analytical coefficient of variation for each amino acid was: leucine 9.0%, isoleucine 7.0% and valine 6.5%.

Sample size, randomization and statistical analysis

Sample size calculations showed that 42 subjects were needed in each group to detect a difference greater than or equal to 1 unit of HOMA-IR, assuming a standard deviation of 1.5 based on previous results in the clinical database of morbid obesity in one of our centres, with a minimum power of 80%. The dropout rate for the study was estimated at 15%. Simple randomization was performed by a statistician not involved in the study using a computer-generated sequence of random numbers for balanced allocation. *Math.random* function was used to generate the random allocation sequence prior to study activation. We did not use any restriction or blocking method. Randomization was assigned using the central database, where the previously reported number generation algorithm was stored. According to eligibility screening by the research co-ordinator, the system generated a unique number that could not be modified or erased.

The treatment arm was not blinded to the participants, care providers or the person assessing the statistical analysis.

Two hundred forty-three patients were assessed initially for the study. Fig. 1 shows exclusion causes and follow-up of participants. Ninety-eight patients were recruited, 44 of whom were assigned to CT and 54 to CPAP. Twelve patients in the CPAP group and six in the CT group discontinued follow-up; therefore, 80 patients completed the study and were analysed.

Continuous variables were shown as mean \pm standard deviation for normally distributed data or median (and interquartile range) for non-normally distributed data, and categorical variables as proportions. The intention-to-treat principle was applied for the analysis of the differences between treatment groups, but missing data were not imputed in order to avoid the dilution effect.

Changes from baseline of treatment with CPAP and conservative treatment were calculated by subtracting the values after the 12-week intervention period from the values before the period; the *t*-test or the Mann-Whitney *U*-test were used. The chi-square test or Fisher's exact test were used for categorical variables. We aggregated patients with IGT and those who screened positive for diabetes in the IGT category, as only three patients proved to be diabetic in the OGTT. Changes after 12 weeks in IGT and MetS were categorized as improved, worsened or unchanged. Spearman's rank correlation was performed for correlating demographic and

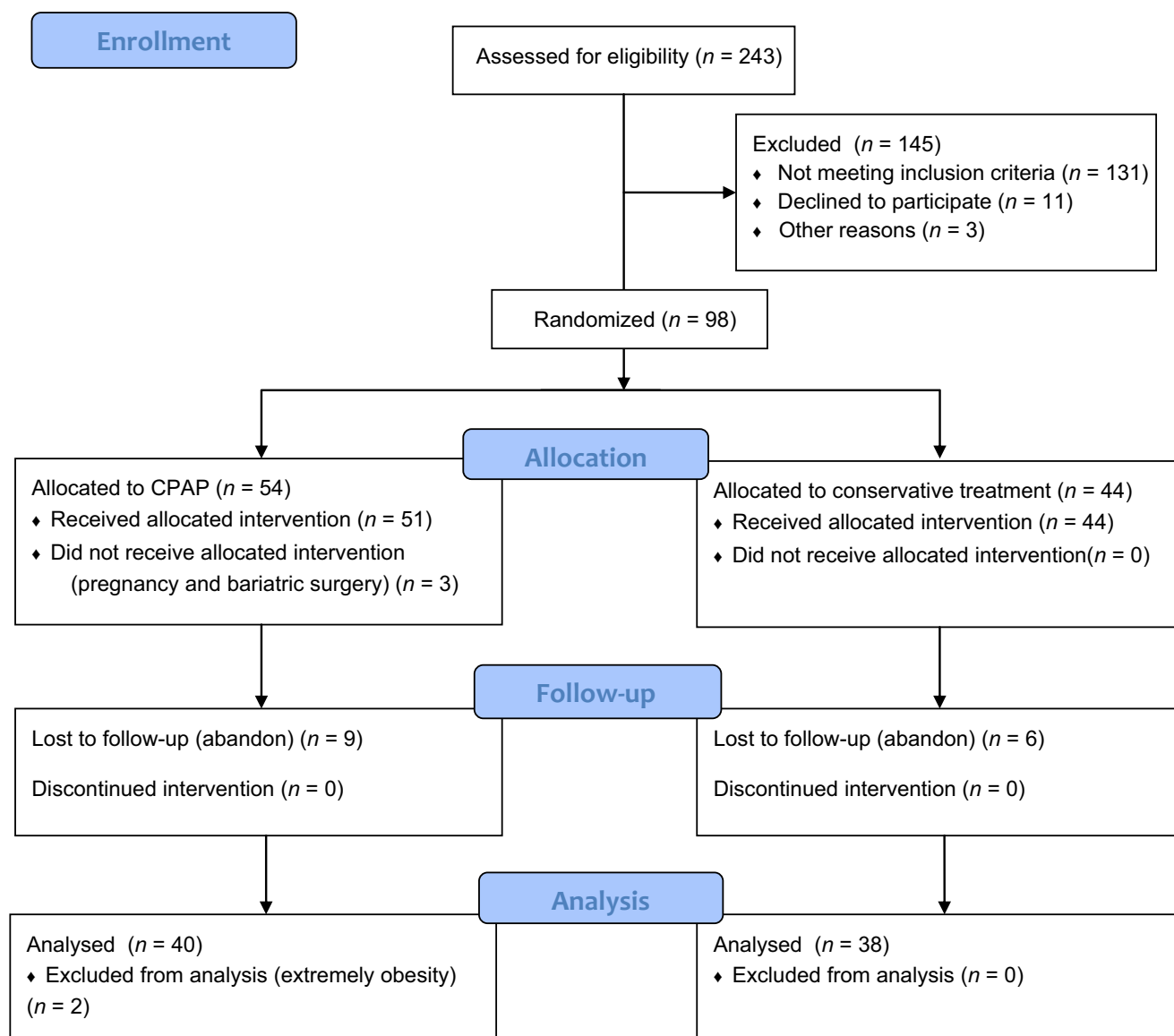


Figure 1. Study flowchart.

sleep data and BCAA concentrations. To determine the possible effect of CPAP on BCAA levels, we performed a multivariate analysis with study group, age, gender, BMI, baseline glucose, HOMA-IR, HbA1c and BCAA levels as the independent variables and post-treatment BCAA levels as the dependent variables.

Two-sided P -values of less than 0.05 were considered to indicate statistical significance. Statistical analysis was performed with the statistical software package SPSS version 19 (IBM Corp., Armonk, NY, USA).

RESULTS

Table 1 shows the baseline characteristics of the CPAP and CT groups. Patients in the CT group had higher BMI and waist circumference when compared to the CPAP group.

There were no major differences in age, sex, OSA severity, glycaemic variables or prevalence of MetS between the two groups.

Changes in BCAA levels after treatment

After 12 weeks of treatment, both groups presented a slight but equivalent weight loss. Most variables explored, including FPG, HOMA-IR and HbA1c, were unchanged in both groups. Metabolic syndrome prevalence were not significantly different between the CPAP and conservative intervention groups. In the CPAP group, IGT reversed in 23.7% and glucose tolerance remained unchanged in 76.3%. No patients worsened in this group. In the CT group, IGT reverted to NGT in 14.7%, remained unchanged in 70.6% and 14.7% developed IGT ($P = 0.039$ at Fisher's exact test) (Salord *et al.*, 2016).

Table 1 Clinical and biochemical characteristics of continuous positive airway pressure (CPAP) and conservative treatment groups at baseline

	CPAP group n = 42	Conservative treatment group n = 38	P-value
Age, years	48.5 ± 8.6	44.6 ± 9.4	0.057
Gender, males n (%)	11 (26)	11 (29)	0.783
BMI, kg m ⁻²	45.7 ± 5	49.3 ± 6.6	0.007
Waist circumference, cm	130 (120–137)	134 (126–147)	0.040
Epworth scale	7.9 ± 4.5	7.9 ± 5.2	0.892
Sleep efficiency, %	82.8 (74.0–87.7)	77.1 (69.3–86.0)	0.321
AHI, episodes h ⁻¹	68.3 (43–88)	52.6 (37–78)	0.276
Time spent <90% of TST, %	13.8 (6–29)	17.7 (6–41)	0.765
ODI 3%, h ⁻¹	57.8 ± 28.0	52.5 ± 28.0	0.402
Metabolic syndrome, n (%)	34 (81)	29 (76.3)	0.613
Fasting glucose, mmol L ⁻¹	5.8 ± 0.70	5.7 ± 0.77	0.533
2 h-PG, mmol L ⁻¹ *	6.7 (5.3–8.9)	6.4 (5.0–7.0)	0.671
HbA1c, %	5.8 ± 0.4	5.7 ± 0.4	0.363
HOMA-IR*	6.8 ± 3.3	6.4 ± 3.6	0.628
Leucine (μmol L ⁻¹)	136 ± 20	140 ± 25	0.563
Isoleucine (μmol L ⁻¹)	74 ± 12	75 ± 16	0.644
Valine (μmol L ⁻¹)	273 ± 37	276 ± 44	0.314

Data are presented as n (%) for categorical data or as mean ± standard deviation or median (interquartile range) for continuous data; *t*-test or Mann–Whitney *U*-test were used for continuous variables and χ^2 or Fisher's exact test were used for categorical variables.

BMI, body mass index; AHI, apnea–hypopnea index; HbA1c, glycosylated haemoglobin; HOMA-IR, homeostasis model assessment–insulin resistance; TST, total sleep time; ODI, oxygen desaturation index; time spent <90% of TST, time spent with SpO₂ <90%.

*2 h-PG (*n* = 35, *n* = 34), HOMA-IR (*n* = 40, *n* = 38).

The mean values of glucose variables and BCAAs (leucine, isoleucine, valine) in the CPAP and the conservative groups are shown in Table 2.

Among plasma BCAAs, significant decreases of leucine levels were observed in both groups when compared with the baseline levels. Finally, because the CT group presented higher levels of obesity than the CPAP group, the analysis was repeated after excluding five patients with extreme obesity. The BMI of the two groups became comparable (45.7 ± 5.1 and 47.5 ± 5.1 kg m⁻²). The decrease in leucine levels remained significant in both groups. We found no apparent modification of the initial results after adjusting for age, gender, BMI, baseline glucose, HOMA and HbA1c levels. Results also remained unchanged after excluding six patients with <4 h of CPAP compliance.

Associations of BCAA levels at baseline

Table 3 shows the correlation coefficients of BCAA levels with anthropometric, sleep and glycaemic variables at baseline of both groups of patients included in the study. Isoleucine levels were associated with HbA1c ($r = 0.287$, $P = 0.011$) and with time spent with SpO₂ <90% ($r = 0.244$, $P = 0.032$).

Relationships between baseline BCAAs and post-treatment metabolic data

In the CT group, a positive correlation was detected between isoleucine and leucine measured at baseline and FPG and HbA1c values measured after treatment (FPG, isoleucine:

$r = 0.463$, $P = 0.004$; leucine: $r = 0.387$, $P = 0.018$; HbA1c, isoleucine: $r = 0.538$, $P = 0.001$; leucine: $r = 0.335$, $P = 0.049$).

Changes in levels of leucine and isoleucine after treatment were related negatively to changes in FPG and HbA1c values only in the CT group (Table 4).

Relationship between BCAA and glucose tolerance

Compared to patients with NGT, patients with IGT had higher levels of isoleucine (78 ± 16 versus 70 ± 13 μmol L⁻¹, $P = 0.014$) and valine (286 ± 36 versus 268 ± 41 μmol L⁻¹, $P = 0.049$), respectively (Fig. 2). In response to treatment, changes of leucine, isoleucine and valine did not differ between patients with IGT and patients with NGT. When this analysis was performed only in patients in which IGT reverted to NGT (nine in the CPAP group and five in the CT group), a significant decrease was observed in leucine levels (141 ± 18 μmol L⁻¹ at baseline versus 122 ± 24 μmol L⁻¹ after treatment, $P = 0.012$).

In patients with IGT, a positive correlation between baseline BCAA levels and HbA1c values measured post-treatment (isoleucine: $r = 0.719$, $P = 0.045$; leucine: $r = 0.755$, $P = 0.031$; valine: $r = 0.755$, $P = 0.031$) was observed in the CT group. No associations were detected between these variables in the CPAP group.

DISCUSSION

In the present randomized controlled intervention study, performed in non-diabetic subjects with morbid obesity and

Table 2 Effect of continuous positive airway pressure (CPAP) and conservative treatment on anthropometric, clinical, glucose and branched-chain amino acids (BCAA) variables

	CPAP group n = 42			Conservative treatment group n = 38			Intergroup differences (95% CI)
	Baseline	12 weeks	Change	Baseline	12 weeks	Change	
BMI (kg m ⁻²)	45.7 ± 5.0	44.7 ± 5.0	-0.96 ± 2.6	49.3 ± 6.6	48.3 ± 7	-0.99 ± 2.0	-0.009 (-1.04 to 1.03)
Waist circumference (cm)	130 (120–137)	129 (119–137)	-2.1 ± 6.8	134 (126–147)	133 (121–141)	-3.3 ± 10.8	-1.2 (-5.18 to 2.18)
Fasting glucose (mmol L ⁻¹)	5.80 ± 0.70	5.60 ± 0.67	-0.13 ± 0.57	5.7 ± 0.8	5.9 ± 2.3	0.205 ± 2.03	-0.33 (-0.10 to 0.40)
Glycosylated haemoglobin (%)	5.8 (5.5–6.1)	5.8 (5.5–6.1)	0.035 ± 0.2	5.7 (5.5–5.9)	5.6 (5.4–6.0)	0.075 ± 0.6	-0.04 (-0.23 to 0.15)
HOMA-IR [†]	6.8 (4.4–8.7)	6.5 (5.2–9.3)	0.32 ± 4.4	6.4 (5.2–11.0)	6.7 (5.1–9.3)	0.044 ± 3.7	0.27 (-1.55 to 2.1)
2 h-PG (mmol L ⁻¹) [†]	6.7 (5.3–8.9)	6.2 (5.1–8.5)	-0.5 ± 1.5	6.4 (5.0–7.0)	6.7 (5.7–7.9)	0.33 ± 1.9	-0.85 (-1.69 to -0.01)*
Leucine (μmol L ⁻¹)	136 ± 20	122 ± 27	-14 (-23 to -5)**	140 ± 25	112 ± 30	-26 (-36 to 2)**	-20 (-26 to -14)
Isoleucine (μmol L ⁻¹)	74 ± 12	72 ± 15	-2 (0 to 10)	75 ± 16	74 ± 16	1 (-3 to 6)	3 (-0.6 to 6)
Valine (μmol L ⁻¹)	273 ± 37	281 ± 38	8 (-7 to 23)	276 ± 44	278 ± 51	2 (-9 to 12)	5 (-4 to 14)

BMI, body mass index; CI, confidence interval; HOMA-IR, homeostasis model assessment–insulin resistance.

**P* < 0.05.

***P* < 0.005.

[†]2 h-PG (*n* = 35, *n* = 34), HOMA-IR (*n* = 40, *n* = 38).

Table 3 Correlation of branched-chain amino acids (BCAA) concentrations with anthropometric, sleep and glucose metabolism variables at baseline

	Valine	Isoleucine	Leucine
BMI	0.008	-0.033	0.001
WC	0.075	0.103	0.186
Epworth scale	0.082	0.203	0.001
AHI	0.160	0.115	0.012
Time spent <90% of TST	0.216	0.244*	0.135
FG	0.153	0.141	0.138
2 h-PG	0.121	0.173	0.007
HbA1c	0.155	0.287*	0.097
HOMA index	-0.010	0.075	-0.042

AHI, apnea–hypopnea index; BMI, body mass index; TST, total sleep time; HOMA, homeostasis model assessment; HbA1c, glycosylated haemoglobin.

**P* < 0.05.

severe sleep apnea, we found that the treatment with CPAP for 12 weeks caused similar changes in circulating BCAA concentrations to CT. However, higher isoleucine and valine concentrations were found in patients with IGT and a differential metabolic response of CPAP and CT was detected between the relationships of BCAAs and glucose homeostasis. Our findings include several important observations.

First, isoleucine levels were associated with HbA1c and time spent with SpO₂ <90%. Obesity-related increases in BCAA levels are associated with dysregulation of BCAA metabolism (Perng *et al.*, 2014). The BCAA catabolic

enzymes are distributed widely in body tissues, and tissue-specific alterations in BCAA metabolism could contribute to elevated plasma BCAAs (Herman *et al.*, 2010; Newgard, 2012; She *et al.*, 2007). Altered systemic BCAA levels have been found to be associated with several parameters for glucose homeostasis, such as HOMA-IR, HbA1c and FPG (Knebel *et al.*, 2016; McCormack *et al.*, 2013; Palmer *et al.*, 2015; Wang *et al.*, 2011). Our results suggest that in subjects with morbid obesity and severe sleep apnea, both obesity and OSA may be relevant in these associations.

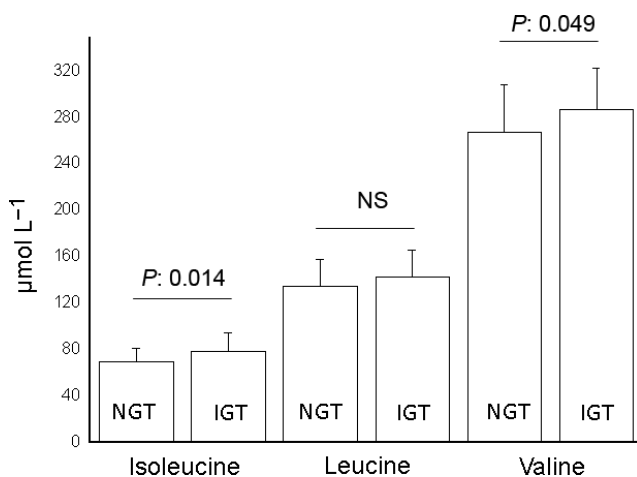
Secondly, a significant decrease in circulating BCAA (leucine) levels was observed in response to dietary or CPAP intervention. BCAAs have showed strong positive associations with type 2 diabetes mellitus risk up to 12 years before onset, and have also been linked to risk of cardiovascular disease. Conversely, several studies have suggested that BCAA values could represent a biomarker of a metabolic dysregulation with the causal metabolites yet to be identified, and facilitate pre-emptive treatments (Ferguson and Wang, 2016), and there is evidence that changes in BCAA levels may correlate with the efficacy of interventions for affecting improvement in metabolic control (Huffman *et al.*, 2011; Kamaura *et al.*, 2010; LaFerrere *et al.*, 2011; Magkos *et al.*, 2016; Shah *et al.*, 2012; Wang *et al.*, 2011). In this study, we hypothesized that changes in BCAA metabolism could mediate in the improvement in insulin sensitivity and glucose tolerance after CPAP treatment in OSA patients. We found that both treatments caused the same decrease in plasma leucine levels. These data do not support the hypothesis that CPAP has independent effects on BCAA

Table 4 Cross-sectional associations for changes in branched-chain amino acids (BCAA) concentrations and changes in anthropometric and glucose metabolism variables

Change	CPAP group n = 42			Conservative treatment group n = 38		
	Leucine change	Isoleucine change	Valine change	Leucine change	Isoleucine change	Valine change
Fasting glucose change	0.114	0.112	0.113	−0.323*	0.173	0.048
2 h-PG change	−0.217	0.145	0.105	0.036	−0.122	−0.015
Glycosylated haemoglobin change	−0.163	−0.099	−0.106	−0.136	−0.409*	−0.121
HOMA-IR change	−0.073	0.136	0.178	−0.226	0.005	0.215
BMI change	−0.035	0.109	0.241	−0 to 147	0.185	−0.025

BMI, body mass index; CPAP, continuous positive airway pressure; HOMA-IR, homeostasis model assessment–insulin resistance.

* $P < 0.05$.

**Figure 2.** Branched-chain amino acid (BCAA) concentrations in patients with normal glucose tolerance (NGT) and impaired glucose tolerance (IGT).

metabolism. However, the relationships among surrogate measures of glucose homeostasis and BCAAs (leucine, isoleucine) differ according to treatment, and our data demonstrate that baseline BCAA levels are correlated with HbA1c and FPG values after treatment only in the CT group. In addition, in response to conservative treatment, changes in levels of leucine and isoleucine were related negatively to changes in FPG and HbA1c values, suggesting that BCAA levels at baseline may be potential contributors in predicting change in FPG and HbA1c after a dietary weight loss intervention and that the greater the BCAA levels at baseline, the smaller the improvement in HbA1c with weight loss. A possible explanation for our findings is that CPAP treatment does not appear to affect changes in BCAA concentrations directly, but it may nevertheless counteract the deleterious associations between baseline BCAAs and measures of diabetes risk, potentially via downstream pathways or alternative mechanisms.

Thirdly, we detected that patients with IGT had higher levels of isoleucine and valine compared to patients with normal

glucose tolerance. Similar changes after treatment were found between patients with IGT and NGT. Therefore, we observed that in patients with IGT, baseline BCAA levels (isoleucine, leucine) correlated positively with after-treatment HbA1c values and negatively with HbA1c change after treatment only in the CT group, suggesting again that the greater the BCAA levels at baseline, the smaller the improvement in HbA1c after a dietary therapy. We did not find any relationships between baseline BCAAs and after treatment glycaemic measures in the CPAP group. Taken together, these observations suggest a role for circulating BCAAs in mediating glucose tolerance in CPAP treatment compared to conservative treatment. The improvement in glucose tolerance observed after 12 weeks of CPAP therapy may reflect the reduction in muscle insulin resistance, and in this context one hypothesis is that BCAA homeostasis may be an intermediate step in modulating muscle insulin resistance with differential functional consequences. However, whether BCAAs are involved in a development of insulin resistance in a functional manner or represent a biomarker of a metabolic dysregulated status is yet to be elucidated (Newbern *et al.*, 2014; Patti *et al.*, 1998; Tai *et al.*, 2010; Wurtz *et al.*, 2013). Our knowledge concerning the biological processes affected by BCAA metabolism and how both systemic and local BCAA metabolism is impaired under pathological stressors such as sleep apnea is largely unknown, and therefore more research is needed to understand these complex biological mechanisms. Our results suggest the potential utility for BCAAs in understanding specific metabolic responses during CPAP treatment, diet-induced weight loss or lifestyle modification in patients with OSA.

Several limitations of the study should be noted. First, concerning the short period of treatment of 12 weeks, a large-scale long-term study is required to confirm our observations. Secondly, we think that the lack of differences between BMI and insulin resistance (HOMA-IR) might be due to the fact that the patients included were extremely obese and highly insulin-resistant, and we cannot draw conclusions about the generalizability of our results.

CONCLUSIONS

In summary, we found that CPAP and dietary intervention caused similar changes in circulating BCAA concentrations. A differential metabolic response of CPAP and conservative treatment was detected between the relationships of levels of BCAAs and measures of glucose homeostasis. Additional studies are needed to determine the interplay between branched-chain amino acids and glucose metabolism and their potential role in the development of insulin resistance and metabolic disorders in patients with sleep apnea.

CLINICAL TRIAL REGISTRATION

The study protocol was approved by both Ethical Committees: Comité Ético de Investigación Clínica de La Fundació de Gestió Sanitària del Hospital de la Santa Creu i Sant Pau de Barcelona and Comité Ético de Investigación Clínica del Hospital Universitari de Bellvitge (PR052/08, 07/064/797). All participants gave their informed written consent. The trial registration number in the ClinicalTrials.gov was NCT 01029561 (available at: <https://clinicaltrials.gov/ct2/show/NCT01029561?term=NCT+01029561&rank=1>).

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AUTHOR CONTRIBUTIONS

AB, DMG, NS and MM conceived and designed the study. AB, DMG, NS, CE, GP and MM supervised the data collection and managed the data, including quality control. AB, DMG, CE and GP provided statistical advice on study design and analysed the data, AB, NS and MM chaired the data oversight committee. AB, DMG, AP and MM drafted the manuscript, and all authors contributed substantially to its revision. AB and MM take responsibility for the paper as a whole.

CONFLICT OF INTEREST

All authors declare no conflicts of interest.

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